

FIG. 1. Sensitivity of the PCR assay. Shown are the results of PCR amplification of the serially diluted *L. donovani* (DD8) DNA analyzed on agarose gels. DNA was extracted from parasite cultures and amplified as described in Materials and Methods. Lane M, 1 kb Ladder (Gibco BRL); lane 1, 10 ng of DNA; lane 2, 1 ng of DNA; lane 3, 10 pg of DNA; lane 4, 1 pg of DNA; lane 5, 10 fg of DNA; lane 6, 1 fg of DNA.

Probe: Ld Ind kDNA

Human DNA: $\xrightarrow{100 \text{ ng}}$

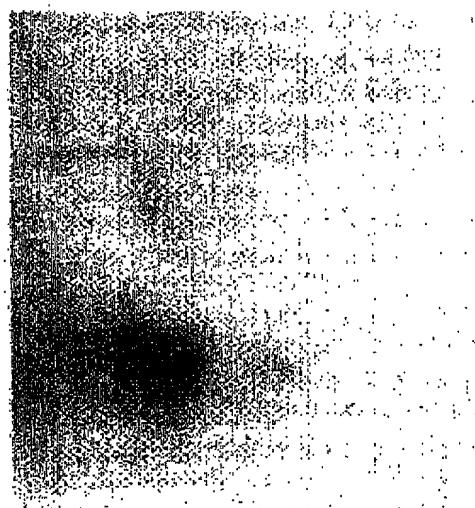
Primer Set: LdI1 & 2

Amt. Ld Ind DNA: $\begin{matrix} 1 \text{ pg} & 0.1 \text{ pg} & 0.01 \text{ pg} & 0 \end{matrix}$

(Kb)

0.87 —

0.6 —



1 2 3 4

FIG. 2. Sensitivity of PCR amplification of *Leishmania* kDNA followed by Southern blot analysis. The PCR contained 100 ng of human genomic DNA and the indicated amount of total DNA from *L. donovani* DD8. The PCR product was probed with parasite kDNA and exposed for about 1 h. Lane 4 represents a PCR containing only human DNA as a control.

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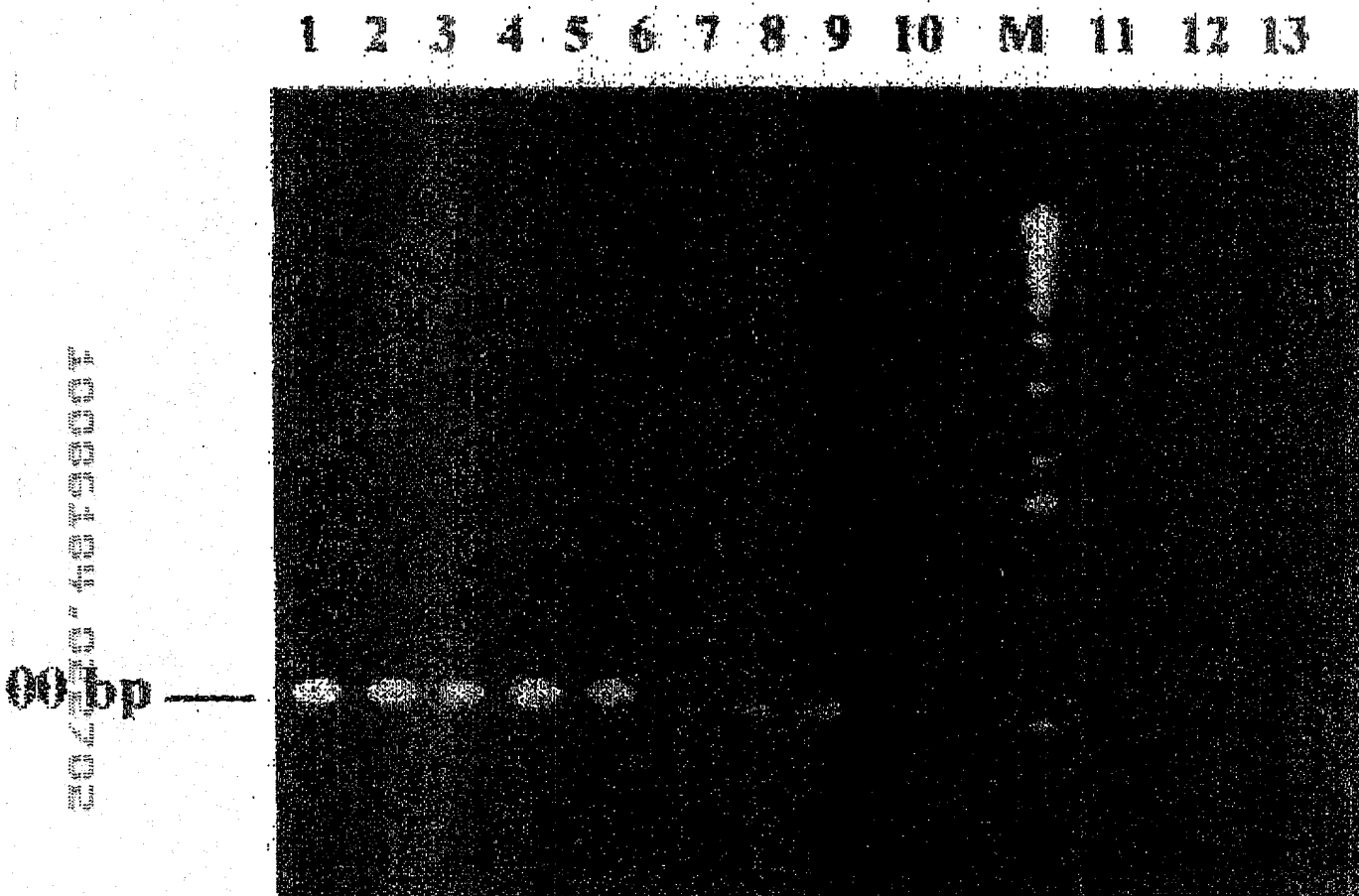


FIG. 3. Amplification of parasite DNA from various strains and isolates of *Leishmania*. DNA (1 ng) isolated from parasite cultures was subjected to PCR and analyzed. Lane 1, *L. donovani* AG83; lane 2, *L. donovani* DD8; lane 3, *L. donovani* HCB8; lane 4, *L. donovani* CB6; lane 5, *L. donovani* HCB 7 (PKIDL origin); lane 6, *L. donovani* 3; lane 7, *L. donovani* WR684; lane 8 *L. donovani* infantum; lane 9, *L. tropica* WR683; lane 10, *L. major* I.V. 39, lane M, 1-kb ladder, lane 11, *Plasmodium*; lane 12, *M. leprae*; lane 13, *M. tuberculosis*.

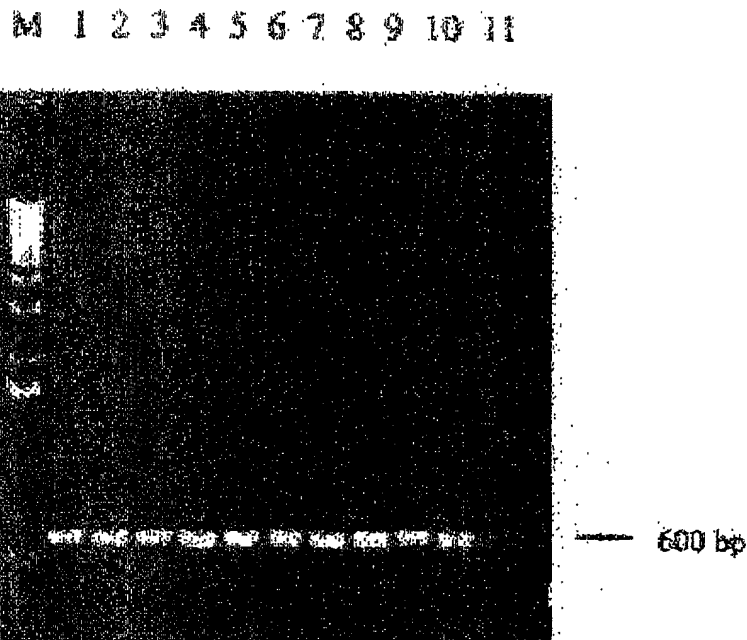


FIG. 4. DNA amplification from recent field isolates of KA and KDL. DNA (1 ng) extracted from cultures of parasite isolates was used for PCR amplification. Lanes: M, 1-kb ladder; 1, KA-1; 2, KA-2; 3, KA-3; 4, KA-4; 5, KA-5; 6, PK-1; 7, PK-2; 8, PK-3; 9, PK-4; 10, PK-5; 11, isolate from a patient with cutaneous leishmaniasis.

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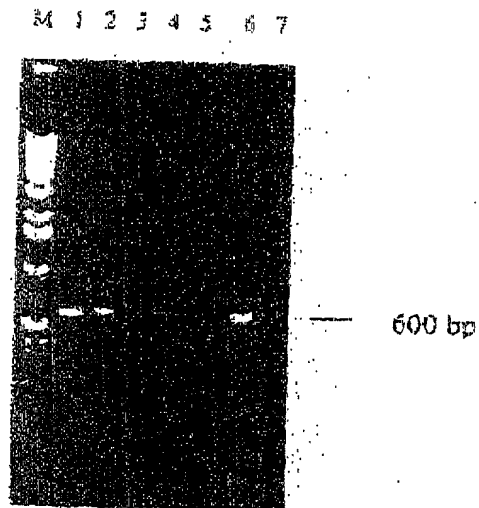


FIG. 5 PCR assay with clinical samples of KA and PKDL DNA (100 ng) isolated from clinical samples was used for PCR amplification. Lane M, 1-kb ladder; lane 1, KA (bone marrow); lane 2, KA (blood); lane 3, malaria (blood); lane 4, tuberculosis (blood); lane 5, control from the area of endemicity (blood); lane 6, PKDL (skin lesion); lane 7, leprosy (lesion).

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Fig 6. Sequence of PCR products with DNA isolated from *L. donovani* DD8 strain, isolates and clinical samples of KA and PKDL.

1 gaattcgccg aaaaatgacc gaaaatgggc caaaaaccca aacttttctg gtccctcggg
 61 tagggggcgtt ctgcgaaaaa cgaaaaatgg gtgcagaaat ccggttcaaa aaatagccaa
 121 aaatgccaaa aatcgggttc gaggcgggaa actggggggtt ggtgtaaaaat agggtcgggt
 181 ggaggggaaa ttgggggttc ggacgtgtgt ggatatggcc tgggtgggga ctttggagt
 241 ggttgtacct gtatgggggt ttggacctgg cttgggggtt ggggttgggt gtgggaaagg
 301 ggtcggcgct atttggagtg acgttggctt ttttgataat tgatatctgc tttaaactgg
 361 attgggttcgg ctggatatac gttgggtcgg ttggatttgg attggatttg gatctgaa
 421 ggggttcggag ggttgacctg gggttgagga gtttgtgggg atagtcttgg algttagtat
 481 ggastgtage ttcctttaat ataaatatta gttggggctg ttgcattagt ttgttcacg
 541 ggagtagcct caggactata ggcgggagat actatattat cggtagtata atactatag
 601 tatacggtae agatataatg taattgtagt atattgtaga tctatgttac agtgtatagt
 661 ctatgaactt actagatata atttgtattt gatgctatag tgctactgat agagtgtacc
 721 tatcaatagt atagaagtat ctgaagctcc ttaaattgggt gggaaatgggt gtgagggctg
 781 gaagagacac cg

201220-1375333